

## Original Article

## CYP2A6 Allele Frequencies in an Iranian Population

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**Background:** Genetic polymorphism of CYP2A6 gene is a major causal factor in the large interindividual differences in nicotine metabolism. It may have an impact on smoking behavior and smoke-related cancer susceptibility. Until now, there are no reports of CYP2A6 allele frequencies in Iranian population.

**Methods:** In the present study, we investigated the frequencies of CYP2A6 alleles in 250 male Iranians. CYP2A6\*2, CYP2A6\*4, CYP2A6\*9, and CYP2A6\*12 were determined by allele-specific polymerase chain reaction.

**Results:** Frequencies of \*2, \*4, \*9, and \*12 alleles were 2.2%, 0.95%, 12.4%, and 1.34%, respectively.

**Conclusion:** These results showed that the distribution of CYP2A6 alleles in Iranian population was different from those reported previously for other ethnic groups. This highlights the importance of conducting further studies to investigate the implications on smoking dependence and cancer in Iranians.

*Archives of Iranian Medicine, Volume 11, Number 6, 2008: 613 – 617.*

**Keywords:** CYP2A6 • Iran • polymorphism

## Introduction

Cytochromes P450 (CYPs), a superfamily of heme-containing mono-oxygenases, are involved in the metabolism of drugs, environmental pollutants, dietary chemicals, and endogenous compounds.<sup>1</sup> Some genes belonging to CYP superfamily were shown to exhibit genetic polymorphisms with varying prevalences of poor metabolizers and extensive metabolizers. Among them is the hepatic isoform CYP2A6, which plays an important role in the metabolism of many xenobiotics. The CYP2A gene family is located on the long arm of human chromosome 19 (19q13.2), assembled in a cluster spanning 350 kb. Three

complete genes (CYP2A6, CYP2A7, and CYP2A13) and two pseudogenes (CYP2A7PC and CYP2A7PT) were identified in this region. Among them, CYP2A6 and CYP2A13 encode active proteins, while CYP2A7 produces a catalytically defective enzyme. CYP2A6, first identified as the human coumarin 7-hydroxylase,<sup>2,3</sup> is responsible for the metabolism of some drugs such as tegafur,<sup>4</sup> methoxyflurane,<sup>5</sup> halothane,<sup>6</sup> disulfiram,<sup>7</sup> and valproic acid.<sup>8</sup> The CYP2A6 gene is highly polymorphic; to date, over 30 alleles have been reported although many are at low frequency or have not been characterized for functional impact.<sup>9</sup> The wild type form of the CYP2A6 gene is termed CYP2A6\*1. Among a variety of alleles, the CYP2A6\*2 (SNP500Cancer ID: CYP2A6-01; 479T>A, L160H), CYP2A6\*4 (whole gene deletion), and CYP2A6\*5 (1436G>T, G479V) alleles are known to cause a lack of enzymatic activity. Also, the CYP2A6\*6 (SNP500Cancer ID:CYP2A6-7; 383G>A, R128Q), CYP2A6\*7 (SNP500Cancer ID:CYP2A6-03; 1412T>C, I471T), CYP2A6\*9 (SNP500Cancer ID:CYP2A6-09; -48T>G), CYP2A6\*10 (1412T>C and 1454G>T, I471T and R485L), CYP2A6\*11

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Accepted for publication: 14 May 2008

(670T>C, S224P), and CYP2A6\*12 (2A6/2A7 hybrid; 10 amino acid substitutions) alleles are known to decrease enzymatic activity. The frequency of these alleles varies substantially among world populations.<sup>10</sup>

Besides the involvement in the metabolism of drugs, CYP2A6 is responsible for a major metabolic pathway of nicotine.<sup>11</sup> The genetic polymorphism of the CYP2A6 gene has been implicated for interindividual differences in nicotine metabolism,<sup>12-14</sup> smoking behavior,<sup>15</sup> and cigarette addiction.<sup>10</sup> Additionally, CYP2A6 is involved in the activation of different tobacco-specific nitrosamines and procarcinogens. A relationship between the genetic polymorphisms of CYP2A6 and smoking-related cancers has also been reported.<sup>16,17</sup> These are of particular interest as ethnic variation in frequencies for CYP2A6 variant alleles exist and may be related to the ethnic differences in nicotine metabolism, smoking behavior, and tobacco-related cancers.

Given that the genetic polymorphism of CYP2A6 possesses some pharmacologic and toxicologic significance, and no data are available regarding the CYP2A6 genetic polymorphism in Iranian population, the aim of this study was to determine the CYP2A6 allele frequencies in Iranian population. Our study was focused on the frequency estimation of alleles \*2, \*4, \*9, and \*12 that are prevalent in Caucasians and Asians/Oriental and have been previously associated with smoking behaviors and/or smoking-related lung cancer.

## Materials and Methods

### Subjects

The study population consisted of 250 unrelated healthy male Iranian individuals from Fars

Province, southern Iran. Their age range was 18 – 36 years with the mean ( $\pm$ SD) age of 36.30 $\pm$ 10.56 years. The participants were smoker or nonsmoker blood donors referring to Iran's Blood Transfusion Center in Shiraz (Center of Fars Province).

They were physically healthy based on history, physical examination, and general health questionnaire (GHQ) administered by a physician. Written informed consent was obtained from the participants. This study was approved by the Medical Ethics Committee of Shiraz University of Medical Sciences.

### Genomic DNA

Peripheral blood samples (8 mL) were collected from the participants by venous puncture. Genomic DNA was extracted from leukocytes by salting out method, as previously described.<sup>18</sup> DNA samples were dissolved in distilled pyrogen free water. DNA concentrations were determined by spectrophotometry at 260 nm.

### Genotyping of CYP2A6 alleles

CYP2A6 alleles were determined by using previously described two-step allele-specific polymerase chain reaction (PCR) assays.<sup>19-22</sup> The first amplification uses genomic DNA as the template with CYP2A6 gene-specific primers and the second amplification uses DNA derived from the first PCR amplification with allele-specific primers. The primers used in this study are shown in Table 1. In each set of samples, three positive controls with different genotyping combinations and a negative control lacking DNA were included. The second amplification products were analyzed on 1.5% or 2% agarose gels containing ethidium bromide. A 100-bp and 1-kb DNA ladder were used for each set of samples to confirm the appropriate amplicon size.

**Table 1.** Primers for CYP2A6\*2, CYP2A6\*4, CYP2A6\*9, and CYP2A6\*12 genotyping assay.

		Primers (5' to 3')	
		Forward	Reverse
CYP2A6*2	First step	GCTGAACACAGAGCAGATGTACA	GGAGGTTGACGTGAACTGGAAGA
	Second step	CTCATCGACGCCCT CTCATCGACGCCCA	TCGTCCTGGGTGTTTCCTTC
CYP2A6*4	First step	GGCCAAGATGCCCTACATG	GGAATAGGTGCTTTTTAAGAATC
	Second step	ACCCACATTAGAAGCTTCTAGA CCCCATTAGAAGCTTCTACTCA	GCTTTTTAAGAATCTGTCTAGAA
CYP2A6*9	First step	ACCTAGACTTAATCTTCCCGTATAC	CCCAAGATCCTGTCTTTCTGAT
	Second step	ATCCTCCACAACAGAAGACCCCTAA	ACGGCTGGGGTGGTTTGCCTTTA ACGGCTGGGGTGGTTTGCCCTTC
CYP2A6*12	First step	GCACCCCTCCTGAGGTACCAC	CGCTCCCCGTTGCTGAATA
	Second step	TGGCTGTGTCCCAAGCTAGGCA TGGCTGTGTCCCAAGCTAGGTG	

**Table 2.** CYP2A6 allele frequencies (%) and genotypes (%) in an Iranian population.

CYP2A6 genotypes	Percentage	Allele	Observed frequency % (±95%CI)
*1/*2	2.8	CYP2A6*2	2.20 (±1.2)
*1/*4	1.4	CYP2A6*4	0.95 (±0.9)
*1/*9	23.1	CYP2A6*9	12.44 (±2.9)
*1/*12	2.6	CYP2A6*12	1.34 (±1)
*2/*2	0.1		
*2/*9	0.84		
*4/*9	0.47		

### Statistical analysis

Allele frequencies were derived by gene counting and using Hardy-Weinberg equation. The Chi square ( $\chi^2$ ) test for goodness-of-fit was used to check whether the distribution of CYP2A6 genotypes in the population deviated from the Hardy-Weinberg equilibrium.

### Results

The CYP2A6 alleles as well as genotype frequencies in the studied group are summarized in Table 2. A summary of the reaction conditions of the two PCRs used to detect the CYP2A6\*2, \*4, \*9, and \*12 alleles is provided in Table 3. Where no \*2, \*4, \*9, or \*12 alleles were detected, the allele was assigned to the default \*1 wild type designation.

The allele frequencies of CYP2A6\*2, \*4, \*9, and \*12 were 2.2%, 0.95%, 12.44%, and 1.34%, respectively. Alleles \*9 and \*4 had the highest and the lowest frequencies, respectively, in our study population. Homozygotes for CYP2A6\*4, \*9, and \*12 alleles were not detected among studied subjects (Table 3). One participant was homozygous for CYP2A6\*2. Three participants were heterozygotes for two genetic variants with decreased (\*9) and lack of (\*2 or \*4) enzyme activity. The frequencies of the genotypes of CYP2A6 alleles were within the 95% confidence interval (CI) estimated by the Hardy-Weinberg equation.

### Discussion

This is a report of CYP2A6 allele frequencies

**Table 3.** Reaction conditions for CYP2A6\*2, CYP2A6\*4, CYP2A6\*9 and CYP2A6\*12 two-step genotyping assays.

Reaction conditions	CYP2A6*2		CYP2A6*4		CYP2A6*9		CYP2A6*12	
	First step (0.3 µg)	Second step (1µ l of first Amplification)	First step (0.3 µg)	Second step (1µ l of first Amplification)	First step (0.3 µg)	Second step (1µ l of first Amplification)	First step (0.3 µg)	Second step (1µ l of first Amplification)
Primer (each) (µM)	0.4	0.5	0.5	0.5	0.5	0.5	0.06	0.5
Each dNTP (mM)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
MgCl <sub>2</sub> (mM)	4	4	3.4	3.4	3	1.4	3.4	3.4
Taq polymerase (units)	2	2	2	2	2	2	2	2
Reaction step								
Initial denaturation, °C (s)	95 (60)	95 (60)	95 (60)	95 (60)	94 (60)	94 (60)	95 (60)	95 (60)
Denaturation, °C (s)	95 (15)	95 (15)	95 (15)	95 (15)	94 (15)	94 (20)	95 (15)	95 (15)
Annealing, °C (s)	60 (20)	50 (20)	52 (20)	50 (20)	55 (30)	68 (40)	58 (30)	65 (20)
Extension, °C (s)	72 (180)	72 (45)	72 (60)	72 (60)	72 (120)	72 (60)	72 (120)	72 (60)
# Cycles	36	20	35	25	35	20	38	25
Final extension, °C (min)	72 (7)	—	72 (7)	—	—	—	72 (7)	72 (7)

in an Iranian population. The study showed that the frequency of distribution of variant allele CYP2A6\*2 in Iranians was 2.2%, which lies well within the ranges reported in previous studies. Previous studies reported a frequency of 1.1 to 3% in European and North-American Caucasians,<sup>19,20,23</sup> and 0.0 – 0.7% in Orientals,<sup>13,20,21,23</sup> for CYP2A6\*2. It is worth noting that the gene deletion mutation CYP2A6\*4, which accounts for 6.7 – 24.2%<sup>13,20,21</sup> of the CYP2A6 alleles in Asians, namely Orientals, had a low frequency (<1%) in Iranian population. Such a low \*4 allele frequency was also reported for Caucasians (0.5 – 1.2%).<sup>20,21</sup>

The frequency of CYP2A6\*9 allele in Iranian population (12.4%) was higher than that found in previous studies on Caucasians (5.2 – 7.2%),<sup>20,22</sup> and lower than those of Orientals (15.6 – 22.3%).<sup>20,22,24</sup> The frequency of CYP2A6\*12 allele in Iranian population (1.34%) was different from that reported for other populations with frequencies ranging from 0.0 – 0.8% in Orientals and to 2.0 – 2.2% in Caucasians.<sup>20,25</sup>

There is no previous report examining the frequency of CYP2A6\*2, \*4, and \*12 alleles in Iranian population. However, only one study examined the prevalence of CYP2A6\*9 allele in Iranians in relation to esophageal cancer.<sup>26</sup> The researchers compared the frequency of \*9 allele in three Iranian groups who were geographically, ethnically, and religiously distinct. A higher frequency of \*9 allele was reported in Turkmans (14%, from northeastern Iran) as compared with those of Turks (5%, from northwestern Iran) or Zoroastrians (4%, from Tehran).<sup>26</sup> The frequency of 12.4% for CYP2A6\*9 allele in the present study was close to that reported for Turkmans (14%), but higher than those of Turks and Zoroastrians. This might be an indication that the difference in \*9 allele frequencies between different Iranian population is related to the differences in their genetic make up rather than their geographic coverage. Further studies are needed to examine the CYP2A6 allele frequencies in different Iranian ethnical groups.

The findings of this study should be viewed in the light of limitation of geographical coverage of the studied population. Nevertheless, the close similarity of the findings of the present study, in terms of the \*9 allele frequency, with that of Turkmans who lived in northeastern Iran might indicate that the variation in allele frequencies is less likely to be geographically determined. Future

studies examining CYP2A6 allele frequencies from different geographical parts of Iran will address this possibility.

In summary, the distribution of CYP2A6 alleles in the Iranian population is different than those of other ethnic groups. These differences highlight the importance of conducting further studies to investigate the implications on smoking dependence and cancer in Iranians.

## Acknowledgment

*This work was financially supported in part by a grant (82-1779) from Shiraz University of Medical Sciences. The authors are grateful to Ms Ewa Hoffmann for the training in CYP2A6 genotyping at the University of Toronto.*

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